PB89-124606

Pesticide Assessment Guidelines, Subdivision O
Residue Chemistry. Series 171-4(c)(3)
Residues in Meat, Milk, Poultry and Eggs
Livestock Feeding Studies

(U.S.) Environmental Protection Agency
Chamblee, GA

Oct 88

U.S. Department of Commerce
National Technical Information Service
NTIS

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. PB89-124606
4. Title and Subtitle: Pesticide Assessment Guidelines, Subdivision O, Addendum 8 on Residues in Meat, Milk, Poultry and Eggs: Livestock Feeding Studies			5. Report Date: October, 1988 6.
7. Author(s): Richard A. Loranger, Ph.D.			8. Performing Organization Rept. No.: 540/09-89-010
9. Performing Organization Name and Address: U.S. Environmental Protection Agency OPP/HED (TS-769C) 401 M St. S.W. Washington, D.C. 20460			10. Project/Task/Work Unit No. 11. Contract(C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address: Same as #9			13. Type of Report & Period Covered 14.

15. Supplementary Notes

ADDENDUM 8 to Pesticide Assessment Guidelines, Subdivision O, Hazard Evaluation: Residue Chemistry (EPA-540/9-82-023 and NTIS Order Number PB83-153981), October, 1982

16. Abstract (Limit: 200 words)

The Data Reporting Guideline (DRG) for Residues in Meat, Milk, Poultry and Eggs: Livestock Feeding Studies, Series 171-4(c)(3), gives guidance to pesticide registrants on the format of their study report so that the Agency can review it efficiently. This guidance clarifies sections in the existing Pesticides Assessment Guidelines, Subdivision O, on data reporting. It provides an outline for the study report and describes the topics which should be addressed such as feed consumption, preparation of the dose, housing and health of the animals, sample collection, sample handling and storage stability, analysis of the samples, submission of representative chromatograms, etc., and provides guidance on the presentation of the results of the study. Data submitters can use the DRG in preparing their reports for submission to EPA to meet 40 CFR 158 requirements for the registration of pesticides.

The DRG for Series 171-4(c)(3), Residues in Meat, Milk, Poultry and Eggs: Livestock Feeding Studies is one of a number of DRG's published by the National Technical Information Service as an addendum to Subdivision O of the Pesticide Assessment Guidelines.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Unclassified and freely available	Unclassified	14
	20. Security Class (This Page): Unclassified	22. Price

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION O

RESIDUE CHEMISTRY

Series 171-4(c)(3)

Residues in Meat, Milk, Poultry and Eggs: Livestock Feeding Studies

ADDENDUM 8 ON DATA REPORTING

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Subdivision O - Residues in Meat, Milk, Poultry and Eggs:
Livestock Feeding Studies

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PESTICIDE ASSESSMENT GUIDELINES

RESIDUE CHEMISTRY

Residues in Meat, Milk, Poultry and Eggs: Livestock Feeding Studies

Subdivision O, Series 171-4(c)(3)

DATA REPORTING

INTRODUCTION

A. Purpose of Feeding Studies

Livestock feeding studies are required under 40 CFR 158.125 when residues of a pesticide requested for registration under the amended Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) appear in livestock feed. Typically, such residues are due to the treatment of agricultural crops (see Table II of the Residue Chemistry Guidelines for feed items associated with each crop[1]) or to the intentional addition of the pesticide to livestock feed (a feedthrough).

The purpose of such studies is to determine the extent to which the pesticide transfers to edible tissues, milk and eggs. Thus, such experiments should answer the question: How much of the "total toxic residue" will occur in meat, milk, poultry and eggs following oral ingestion of the pesticide? ("Total toxic residue" refers to the parent pesticide plus its degradation products, metabolites and impurities of toxicological concern which are usually determined by a separate metabolism study using radiolabeled material.) If the data show that finite residues are expected in animal products from the proposed use of the pesticide, the studies can be used to determine appropriate tolerances.

B. Objective of Data Reporting Guideline

Meat, milk, poultry and egg feeding studies are discussed in Subdivision O of the Pesticide Assessment Guidelines: Residue Chemistry. This Data Reporting Guideline (DRG) is supplemental to the Guidelines and designed to aid registrants in conducting and reporting the results of livestock feeding studies. Although it is not mandatory, following this DRG will help the registrant collect and organize complete data in a format that facilitates Agency review. Registrants are also advised to refer to our Standard Evaluation Procedure (SEP)[2] for details on the considerations involved in review of such data.

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[1] Pesticide Assessment Guidelines, Subdivision O: Residue Chemistry, published by the National Technical Information Service (NTIS) as PB#83-153981.

[2] To be published.

This DRG pertains to organizing and presenting the substance of the data report. PR Notice 86-5, effective November 1, 1986, available from the Office of Pesticide Programs, US EPA, pertains to physical formatting of reports (which are referred to as "studies") and submittal packages. Some of its requirements are mandatory.

RESPONSE TO PUBLIC COMMENTS

The purpose of this section is to acknowledge and address comments received by the Agency in response to the public notice in the FEDERAL REGISTER, Volume 53, No. 101, p. 18896, May 25, 1988. The Agency has reviewed the submitted comments on this Data Reporting Guideline (DRG). This addendum to the Pesticide Assessment Guidelines (Subdivision O) is not intended to create new data requirements, but to provide for consistent organized reporting of livestock feeding studies.

Submitted comments are listed below followed by the Agency's response.

1. Comment:

With respect to sample collection (III. B. 1.), AM and PM milk are usually composited in the ratio of production. This is a more realistic assessment of the RAC as most dairy operations ship one time per day.

Response:

We do not object to such compositing of the milk from each individual cow. However, as stated in the Subdivision O Guidelines, "Milk from animals within a dosage group should not be pooled...." We have revised the DRG to clarify this point.

2. Comment:

Under Analysis of Samples (III. D. 4.), "Tabulation of analytical results and example calculations should be sufficient. Volumes of 'raw' data are available for study audit."

Response:

We did not intend for "volumes" of raw data to be submitted. The information we are requesting (sample weights, final extract volumes, volumes of aliquots injected, peak heights/areas, etc.) could be presented for all samples in a table occupying a few pages. We believe sufficient data should be available to verify the residue level for any sample in the study. With respect to chromatograms, the DRG notes that representative ones should be supplied for each matrix (the latter phrase has been added for clarification).

3. Comment:

With regard to "V. CONCLUSIONS", the proposal of tolerances is not within the scope of the report and is handled through section F. Tolerances supported by the data may change as more crops are added. It would be better to address the question of transfer factor in the livestock feeding

report. This factor can be used when feed items are registered to determine appropriate tolerances for animal commodities.

Response:

We agree that meat and milk tolerances often increase as more feed items are registered for a pesticide. A discussion of the extent of transfer is a reasonable suggestion. The "CONCLUSIONS" section has been revised to incorporate this comment.

4. Comment:

The potentially large range of dietary burdens as more feed items are registered makes selection of feeding levels difficult. The question arises as to what is "X" when referring to the recommended doses of 1X, 3X and 10X. "A more reasonable approach for selection of feeding levels would be to cover the expected range of dietary burdens and perhaps a 2X of the maximum anticipated feeding level. For example a dose range of 1, 10, 50 and 100 ppm might be appropriate for a product which is expected to be used on soybeans [initial tolerance submission with low residues] and alfalfa [future anticipated use with high residues]. The rationale for selection of these feeding levels should not need to be presented in the livestock feeding report."

Response:

We agree that in some cases the determination of feeding levels is difficult and involves considerations beyond the initial petition for a tolerance on a feed item. For this reason we do request that an explanation of the doses employed in the study be presented. We do note that the "1X, 3X, 10X" dosing scheme is not written "in concrete," but a suggested range to cover the use of immediate interest plus other feed items that may be added in the future. Other combinations of doses (such as the one provided by the commenter for soybeans and alfalfa) are often acceptable provided a satisfactory rationale is given. We are keeping the request for such rationales in the DRG, but have revised the paragraph (III. A. 1.) on dosing to reflect that additional dose ranges may be adequate.

GUIDELINE

The report should include all the information needed to completely describe the administration of the test material (pesticide and/or its metabolites) to the animals (the in-life or feeding portion of the study); the collection, handling, and storage of samples; and analyses of the latter for the residue of concern. The results of the study and conclusions concerning the extent of transfer of residues should also be furnished.

Often data may be more clearly presented in tables or figures, which may be included in Sections II-V where they are discussed or grouped together under a separate heading. As guidance for when this is appropriate, data requirements that are best submitted as a table or figure are marked with an asterisk (*) in this guideline.

The following format is suggested for the report:

COVER PAGE

Title page and additional documentation requirements (i.e., requirements for data submission and statement of data confidentiality claims) if relevant to the study report, should precede the content of the study formatted below. These current requirements are described in PR Notice 86-5 published by the Office of Pesticide Programs on July 29, 1986.

TABLE OF CONTENTS

The table of contents should provide page numbers on which are found the essential elements of the study, to include the following: Introduction and Summary, Materials, Methods, Results and Discussion, Conclusions, Tables/Figures (flowsheets, etc.), Certification, References, and Appendices. The requirements of each of these sections are discussed below.

I. INTRODUCTION AND SUMMARY

A. This section should provide background and historical perspective for the study. It should include:

1. Registration history;
2. Proposed use of the pesticide;
3. Purpose of the study; and
4. Summary of the results.

B. The summary of the experiment should include:

1. A discussion of any unusual problems encountered and how these were resolved;

2. A discussion of any deviation from the experiment's protocol and the effect this may have had on the results; and

3. A brief description of the study's findings addressing such questions as;

a. Do residues transfer,
b. Preferential accumulation in certain organs,
c. Highest residues, and
d. When did residues plateau?

C. A comparison of the results to those of the animal metabolism studies would also be useful.

II. MATERIALS

A. Test Substance

1. The pesticidal active ingredient and/or its metabolites which are fed should be identified by:

a. Chemical name;
b. Common name (ANSI, BSI, ISO);
c. Company developmental name/number; and
e. Chemical Abstracts Service (CAS) number.

2. The source and purity of each compound should be specified.

3. Chemical structures* of these compounds are also desired.

4. The rationale for choosing the test material should be presented with emphasis on any differences between it and the residues expected on feed items.

B. Test Facilities

The animals' housing should be described. Factors to consider include:

1. Sizes of enclosures;
2. Individual versus group housing;
3. Food and water containers;
4. Temperature;
5. Lighting; and
6. Waste handling.

C. Test Animals

1. A description of the test animals should include:

a. Species;
b. Breed;
c. Age;
d. Weight; and
e. Health status.

2. The number of animals per feeding level must be specified.

3. The mode of identification should be noted (e.g., ear tags).

4. Body weights* and egg/milk production* should be reported for both the acclimation and dosing periods.

5. Any health problems, abnormal behavior, or unusual treatment of animals should be reported and the effect of these on study results discussed.

D. Feed

1. The animals' diet during acclimation and the dosing period should be described as to both:

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* Material which would best be presented in a table or figure. Refer to section VI of this DRG.

a. The types of feed (e.g., corn grain, layers mash, alfalfa pellets) and liquids; and

b. The quantities provided (i.e., specific amounts or ad libitum).

2. Feed consumption* (dry weight) should be reported on an individual or treatment group basis throughout the study.

III. METHODS

A. Dosing

1. The preparation of the dose should be described (mixing with feed or concentrate ration, gelatin capsule, bolus, etc.). The ppm level of the test material in the total diet (dry weight basis) is needed. The recommended doses are 1X, 3X and 10X the anticipated dietary intakes from proposed usages of the pesticide. The calculation of these dietary burdens based on Table II of the Residue Chemistry Guidelines[3]/ should be explained. The petitioner should consider possible future uses of the pesticide when determining the dosages to be fed. Dosing schemes other than 1X, 3X, and 10X are acceptable provided a satisfactory rationale is given.

2. The date of dose preparation should be specified along with the storage conditions prior to its administration.

3. A brief description of the method used to analyze spiked feeds and the results of such analyses should be presented. These analyses should demonstrate that the pesticide was stable in the feed or dosing material throughout its entire storage period.

4. The frequency of dosing should be reported if the test material is not incorporated into the total diet or feed.

5. The dates of the initial and final doses (or the total length of the dosing period) should be indicated.

B. Sample Collection

1. The collection of milk and eggs should be described with any differences from normal practice explained. Any compositing or pooling of samples ought to be noted, although milk from animals within a dosage group should not be pooled. Compositing the AM and PM milk from each individual cow in the ratio of production is acceptable.

2. The collection dates* for those samples which are analyzed for the residue of concern should be reported.

3/ Pesticide Assessment Guidelines, Subdivision O: Residue Chemistry, published by NTIS as #PB83-153981.

3. The mode of sacrifice and the time interval in hours between the latter and the administration of the last dose should be specified. An explanation of intervals longer than 24 hours should be presented along with a discussion of their effect on residues.

4. The tissues taken after sacrifice, their type (e.g., thigh muscle, omental fat, etc.), and their weights should be listed. Combining of samples from different animals should be noted (usually acceptable for poultry, but not ruminants).

C. Sample Handling and Storage Stability

1. The storage and handling of tissues, eggs and milk between sample collection and analysis should be described. Factors to consider are:

   a. Sample preparation (e.g., chopping) prior to storage;
   b. Containers;
   c. How quickly the samples are put into storage;
   d. Storage temperature;
   e. Length of storage (dates of collection, shipping, analysis, etc.); and
   f. Mode of shipping, if applicable.

2. Evidence should be presented showing that the storage did not affect the results of the study. Preferably, this is obtained by concurrently spiking control samples and storing them under the same conditions as samples from treated animals. For guidance in this area refer to the separate Data Reporting Guideline on a Storage Stability Study.[4]/ If such information is provided in another section of the overall data package, the study may be referenced.

D. Analysis of Samples

1. A detailed description of the analytical method employed to measure residues should be provided along with a statement as to which chemical species were measured (parent pesticide, metabolites). When the method has been submitted as a separate report in the total data package (as is often the case), it may simply be referenced. See the separate Data Reporting Guideline on Analytical Method(s)[5]/ for assistance on how to describe methodology.

2. Recovery data should be obtained concurrently with the residue analyses to validate the method and establish its sensitivity (lowest reliable quantitation limit). The experimental design of these validation studies should be described including:

   a. Identity of the test compounds and substrates (tissues, milk,

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[4]/ Published by NTIS as #PB86-248192.

[5]/ ibid.

and eggs);
b. Magnitudes of fortification levels;
c. Number of replicates per test compound per level; etc.

3. Dates* of sample fortification, extraction, and analysis of extracts should be listed. If extracts are not analyzed on the day of preparation, storage conditions should be described.

4. Raw data* such as sample weights, final volumes of extracts, and peak heights/areas should be furnished for control, fortified (including those for storage stability data) and treated samples to support reported residue values and recoveries. Analytical responses of standards (calibration curves*) are also needed.

5. Representative chromatograms* should be supplied for control, fortified, and treated samples of each matrix (milk, eggs, each edible tissue, etc.) along with a few sample calculations of residue levels and percent recoveries using the raw data.

IV. RESULTS AND DISCUSSION

A. Recovery percentages* (all values, not just averages or ranges) for the pesticide and/or its metabolites should be reported for tissues, milk, and eggs fortified with these compounds.

B. Storage stability data* showing the behavior of residues as a function of time in tissues, milk and eggs should be submitted. Storage duration and temperature of these samples should be specified.

C. Levels* of the "total toxic residue" should be reported for each tissue for each feeding level including control (untreated) samples. The tissues recommended for analysis include muscle, fat, liver and kidney (latter not required for poultry). The individual values should be listed for all samples (not merely averages or ranges). It should be clearly indicated whether or not residues have been corrected for recoveries. If the parent pesticide and its metabolites are measured separately, the residues of each should be reported.

D. Residues* in milk and eggs should be listed for each feeding level including controls along with the dates of sample collection. As with tissue residues, the values for each sample should be reported (not just ranges or means).

E. Discussion should be presented as to whether the data indicate that residues of the pesticide transfer to tissues, milk and eggs. If so, when did residues plateau in milk and eggs? Do they preferentially accumulate in certain tissues? Are the results consistent with the ^{14}C metabolism studies?

V. CONCLUSIONS

A conclusion must be reached as to whether residues of the pesticide transfer from feed items to meat, milk, poultry and eggs. If so, the

extent of transfer should be discussed. The results can be summarized by a table* showing either the ranges or maximum residues in each type of sample for each feeding level. Such a table could then be used to determine appropriate tolerances each time additional feed items are registered.

VI. TABLES AND FIGURES

NOTE: This section need only include those tables or figures not included in Sections II-V.

A. The following data should be presented in tabular form:

1. Vital statistics of the test animals throughout the study including body weights, egg or milk production, and feed consumption.

2. Dates of sample collection, fortification, extraction and analysis.

3. Raw data such as responses of standards, sample weights, final volumes of extract, volumes of aliquots injected, and peak heights/areas for all control, spiked (including storage stability) and treated samples.

4. Recoveries of parent compound and/or its metabolites from tissues, milk and eggs.

5. Residues of parent pesticide and/or its metabolites in storage stability samples as a function of time.

6. Levels of the "total toxic residue" in tissues, milk and eggs from both treated and untreated (control) animals.

B. The following should be presented as figures:

1. Chemical structures and names of compounds which are fed to test animals and of those which are measured in tissues, milk and eggs.

2. Reproductions of representative chromatograms (gas, high liquid performance, thin layer, etc.) for control, fortified and treated samples and of any other graphic data (e.g., mass spectra, calibration curves, plot of egg/milk residues as a function of time, plot of residues versus time for storage stability samples, etc.) essential to the study.

VII. CERTIFICATION

Certification of authenticity by the Study Director (including signature, typed name, title, affiliation, address, telephone number and date).

VIII. REFERENCES

Any references cited in the report should be included here.

IX. APPENDICES

Reproductions of published reports that support the submitted study may also be included here if in the registrants opinion, it will increase the efficiency of its review by the Agency.

* Material that would best be presented in a table or as a figure.